of various molecules and signaling pathways in mouse mesendoderm formation and axial elongation morphogenesis.

doi:10.1016/j.ydbio.2010.05.207

Program/Abstract # 167
BMP signaling through ACVR1 is crucial for establishment of the left–right asymmetry via proper formation of node cilia in the mouse

Yuji Mishina, Vesa Kaartinen, Yuji Mishina
Dept of Biologic and Materials Sciences, School of Dentistry, Univ. of Michigan, Ann Arbor, MI, USA

Bone morphogenetic proteins (BMPs) have multiple functions including their role in the establishment of left–right patterning in vertebrate development. Recently, we discovered that BMP type I receptor Acvr1 in the epiblast is required for proper left–right patterning. To address further how Acvr1 is involved in the process, we utilized a conditional gene inactivation strategy to rescue the gastrulation defects of the Acvr1 null mutation. Mosaic inactivation of Acvr1 mutants in the epiblast by using Mox2-Cre (Acvr1:Mox2-Cre) resulted in abnormal heart looping and bilateral expression of left side markers in the lateral plate mesoderm. The mutant embryos displayed an abnormal cilia development that resulted in a defect of a cilia-driven leftward fluid. Complete inactivation of Acvr1 in the epiblast by using Sox2-Cre resulted in complete lack of cilia in the node. Expressions of intraflagellar transport genes, which are important for node cilia formation, were downregulated. Interestingly, Acvr1:Mox2-Cre embryos displayed abnormal cilia development in a ventral part of the neural tube. Mouse embryonic fibroblasts deficient in Acvr1 fail to form the primary cilium, when stimulated by serum starvation. These suggest that ACVR1 is essential for proper development of primary cilia by regulating cell cycle progression to establish a left–right asymmetry at the node.

doi:10.1016/j.ydbio.2010.05.208

Program/Abstract # 168
Functional analysis of the mouse Nodal antagonist, Cerl2, during left–right axis formation

Jose M. Inacio, Sara Marques, Jose A. Belo
Centre for Molecular and Structural Biomedicine, Univ. of Algarve, Faro, Portugal

Although recently our understanding of how the LR asymmetry is generated in vertebrate embryos has seen rapid progress, many important questions remain to be explained. In mouse embryos, the leftward flow of the extra-embryonic fluid in the node cavity, called nodal flow, seems to be the symmetry-breaking event. However, it is not yet know how this flow functions or how the asymmetric signal(s) generated in the node is/are transferred to the lateral plate mesoderm. The mouse gene *cerberus-like2(cerl2)* encodes a 20-kDa protein with a predicted signal peptide sequence and a cysteine-rich domain (CRD) containing nine cysteines characteristic of the Cerberus/DAN family. Whole-mount *in situ* hybridization studies showed that *cerl2* transcripts could be first detected in a horseshoe-shaped expression pattern in the perinodal region of the mouse embryo (E7.0), resembling *Nodal* expression at this stage. At stage E7.5, expression of *cerl2* begins to decrease in intensity on the left side, and by early somitogenesis (E8.0), it can be strongly detected in the right side of the node, assuming a complementary expression pattern to that observed in Nodal. Furthermore, it was shown that Cerl2 activity is upstream of the Nodal receptor inhibiting Nodal and its downstream targets. A physical interaction between these two proteins exists, which suggests that Cerl2 is a secreted Nodal antagonist. Here, to elucidate the role of Cerl2 protein in the early events of symmetry breaking the functional activity of this Nodal antagonist will be discussed.

doi:10.1016/j.ydbio.2010.05.209

Program/Abstract # 169
Comparative proteomic analysis of the left and right sides of chick embryos

Yonghua Zhang[a,b], Annie Simard[b], Aimee K. Ryan[a,b]
[a]*Department of Human Genetics and Pediatrics, McGill University, Montreal, Canada*
[b]*The Research Institute of the McGill University Health Center, Montreal, Quebec, Canada*

In vertebrates, it is critical for normal physiological function that the internal organs are asymmetrically positioned with respect to the midline. The chick embryo has been extremely useful to identify many of the molecules that direct patterning of the left–right (L–R) axis during embryogenesis. The asymmetric expression of several of these molecules is regulated at the level of transcription. Here, we investigated the proteomic profiles of the left and right sides of HH stage 8/9 chick embryos to identify proteins that are asymmetrically expressed and required for normal patterning of the L–R axis. Protein extracts from biological triplicates were separated on SDS-PAGE, subjected to tryptic digestion and then analyzed by ion-trap tandem mass spectrometric analysis. 248 proteins were identified in all samples from the left side and 237 proteins were identified on the right side. Of these proteins, 23 were found to be left-enriched and 7 candidates were right-enriched, exhibiting a 1.5-fold or greater change between the left and right sides. We are currently confirming the asymmetric expression of these proteins by western blot analysis and immunohistochemistry. The results of these studies will be presented.

doi:10.1016/j.ydbio.2010.05.210

Program/Abstract # 170
Left–right determination requires endoderm function in mice

Ranajeet S. Saund[a], Masami Kanai-Azuma[b], Yoshiakira Kanai[c], Yukio Saijoh[a]
[a]*Dept. of Neurobiology and Anatomy, University of Utah, Salt Lake City, USA*
[b]*Tokyo Medical and Dental University, Japan*
[c]*University of Tokyo, Japan*

The asymmetric placement and morphogenesis of visceral organs along the left–right (LR) axis is crucial for proper embryonic development. The development of LR asymmetry requires symmetry breaking at the node and the left-side specific expression of the TGFβ ligand, *Nodal* in the lateral plate mesoderm (LPM) as a left-side determinant at E8.5. Our previous work has shown that *Nodal* expression in the node is required for the expression of *Nodal* in the left LPM, although the mechanism by which the asymmetric signal generated at the node is transferred to the LPM is still unknown. To test the significance of definitive endoderm in LR asymmetry, we have investigated the role of Sox17, a HMG-box transcription factor required for endoderm differentiation in mice. The *Sox17* expression pattern suggests that endoderm may be involved in node formation and LR signal transfer. Analysis of *Sox17* mutant embryos showed abnormal node morphogenesis and absence of *Nodal* expression in the LPM, suggesting that endoderm differentiation is required for LR asymmetry. The reduced node size and altered protein localization in